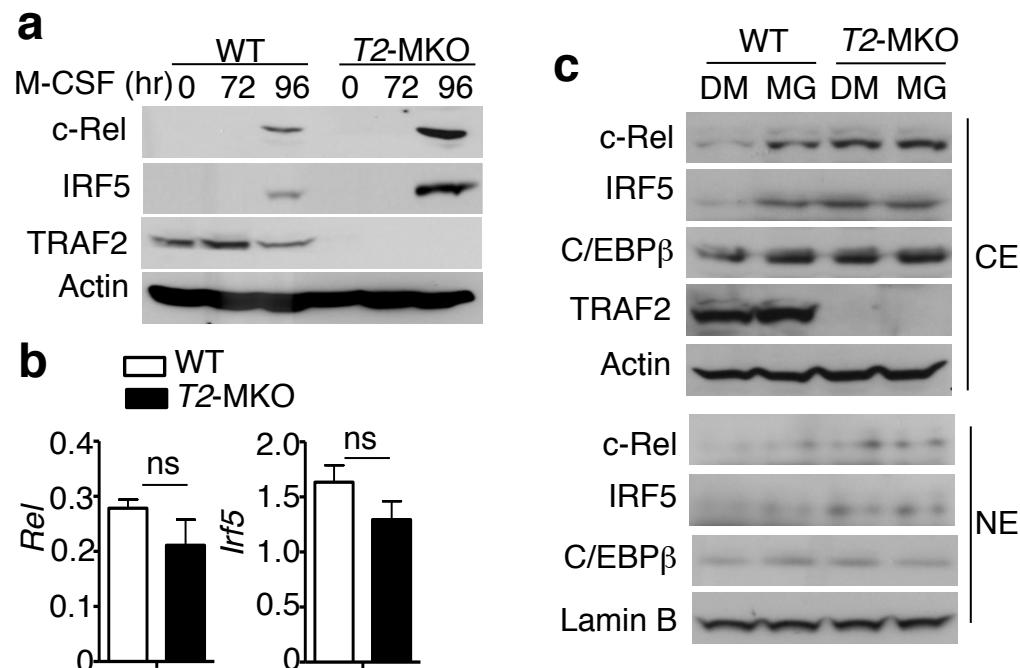
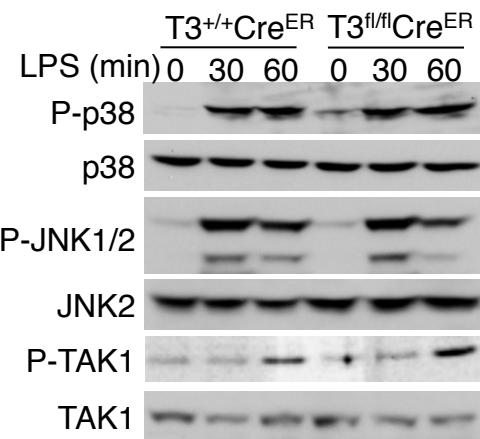


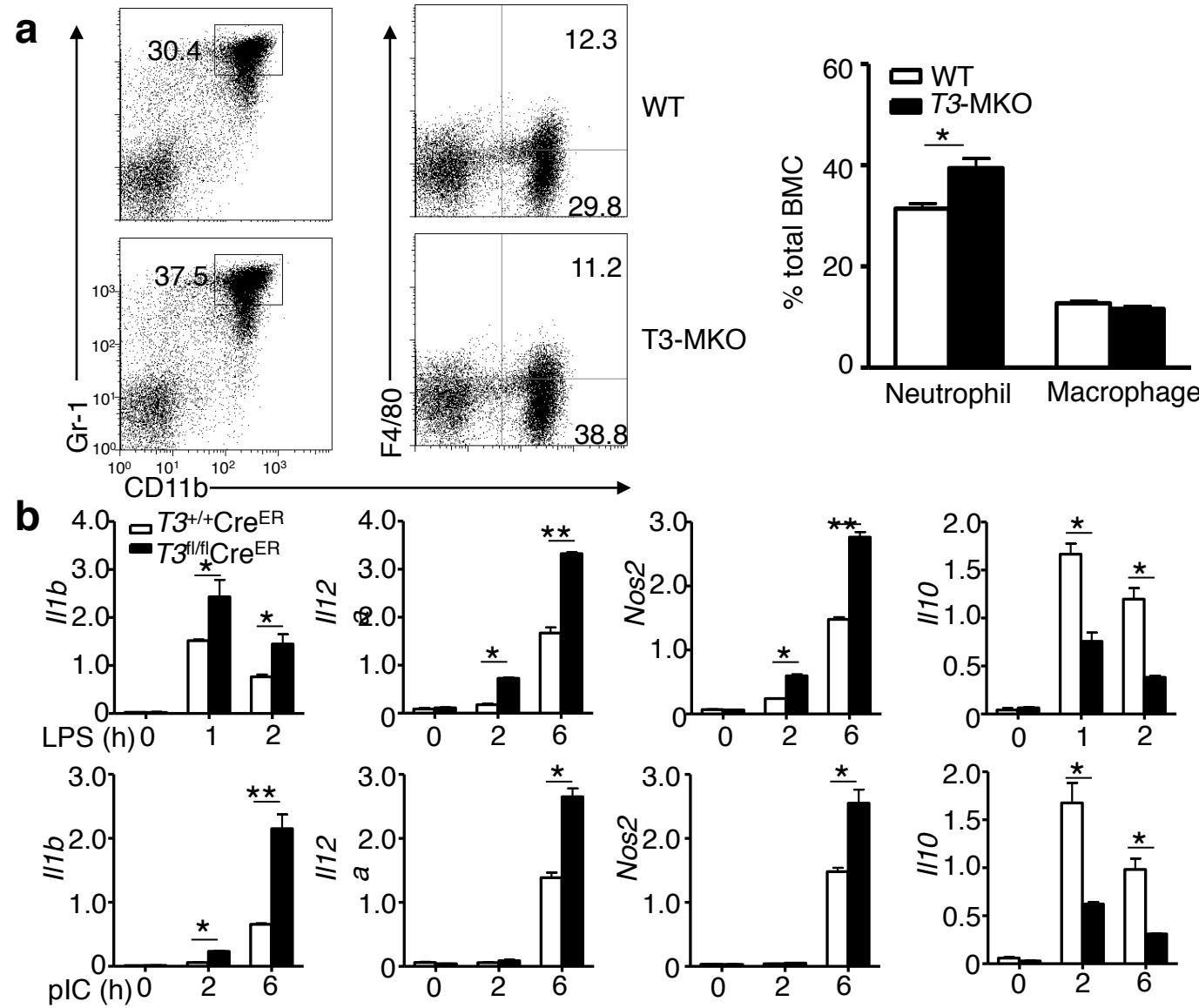
Supplementary Figure 1. Myeloid cell-specific TRAF2 ablation does not affect macrophage differentiation but promotes proinflammatory cytokine induction by poly(I:C) and IL-1 β . (a) Flow cytometry analysis of the percentage (numbers in quadrants) of neutrophils ($CD11b^+Gr-1^+$) and macrophages ($CD11b^+F4/80^+$) in the bone marrow cells (BMC) of WT or *TRAF2*-MKO mice. Data are presented as a representative FACS plot (left) and summary graph based on 3 animals of each genotype (right). (b-d) qRT-PCR analysis of the indicated mRNAs in WT and *TRAF2*-MKO BMDMs (b,d) or peritoneal macrophages (c) stimulated with poly(I:C) (b,c) or IL-1 β (d). Data are representative of three or more independent experiments. The error bars represent standard deviation (SD). Differences between experimental and control groups were determined by Student's *t* test. *P < 0.05; **P < 0.01.



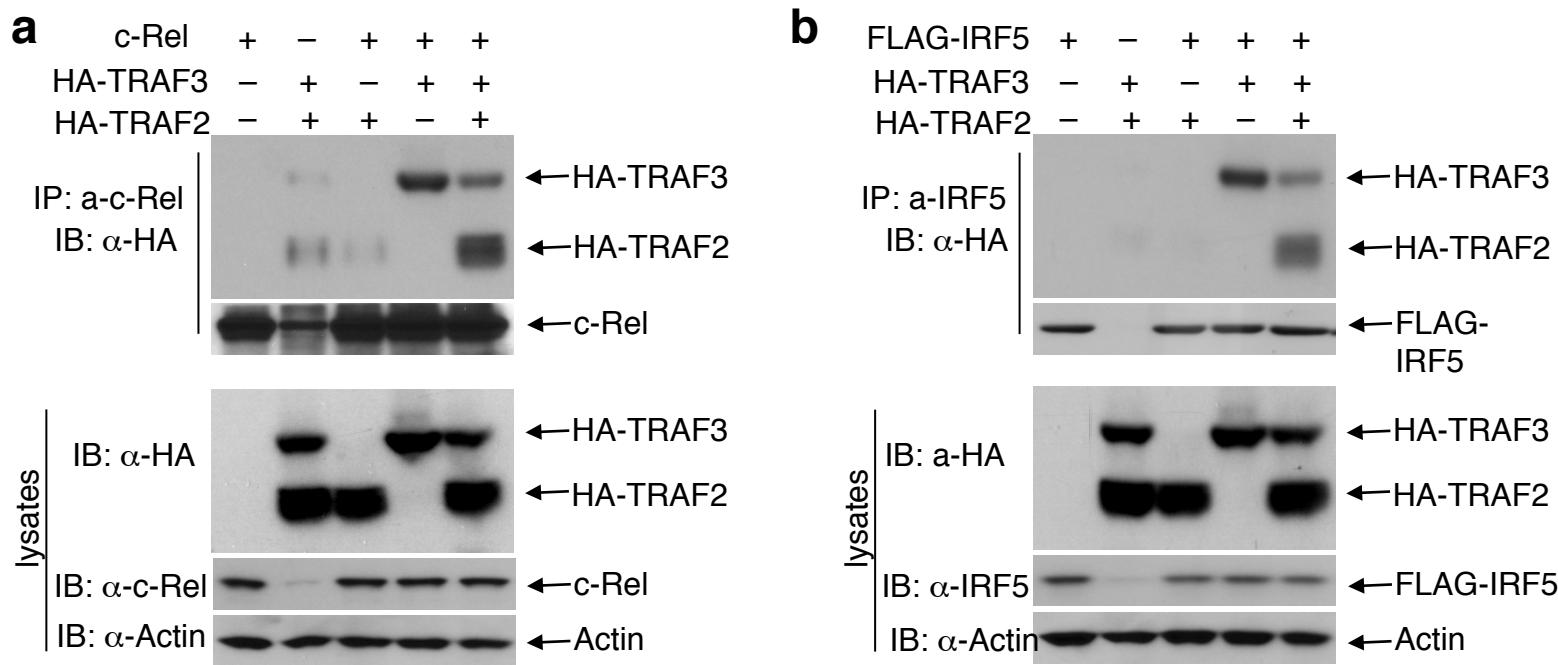
Supplementary Figure 2. TRAF2 mediates degradation of c-Rel and IRF5 in the cytoplasm of macrophages. (a) IB assays using whole-cell lysates of WT or *Traf2*-MKO bone marrow cells, stimulated with MCSF for the indicated time points. (b) qRT-PCR analysis of *Rel* and *Irf5* mRNA levels (fold relative to the internal control *Actb* mRNA) in WT and *Traf2*-MKO BMDMs. (c) Immunoblot analysis of the indicated proteins in the cytoplasmic (CE) and nuclear (NE) extracts of WT and *Traf2*-MKO BMDMs treated with DMSO (DM) or MG132 (MG) for 1hr. The error bars represent standard deviation (SD). Differences between experimental and control groups were determined by Student's *t* test.



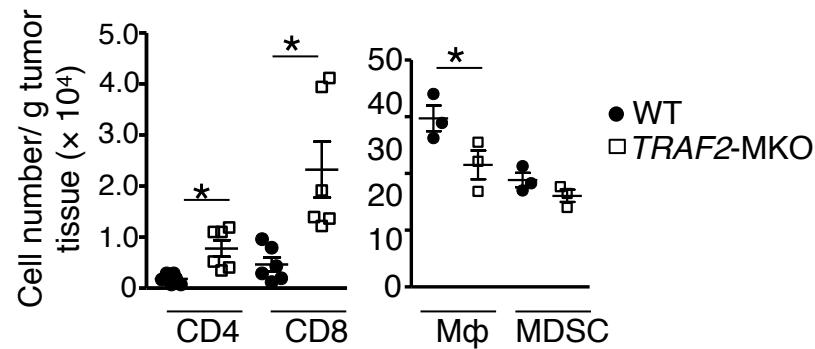
Supplementary Figure 3. TRAF3 deficiency does not promote LPS-stimulated phosphorylation of p38 or JNK. IB analysis of the indicated phosphorylated (P-) and total proteins in whole-cell lysates of LPS-stimulated Traf3^{+/+}Cre^{ER} or Traf3^{f/f}Cre^{ER} BMDMs differentiated in the presence of the Cre inducer tamoxifen.



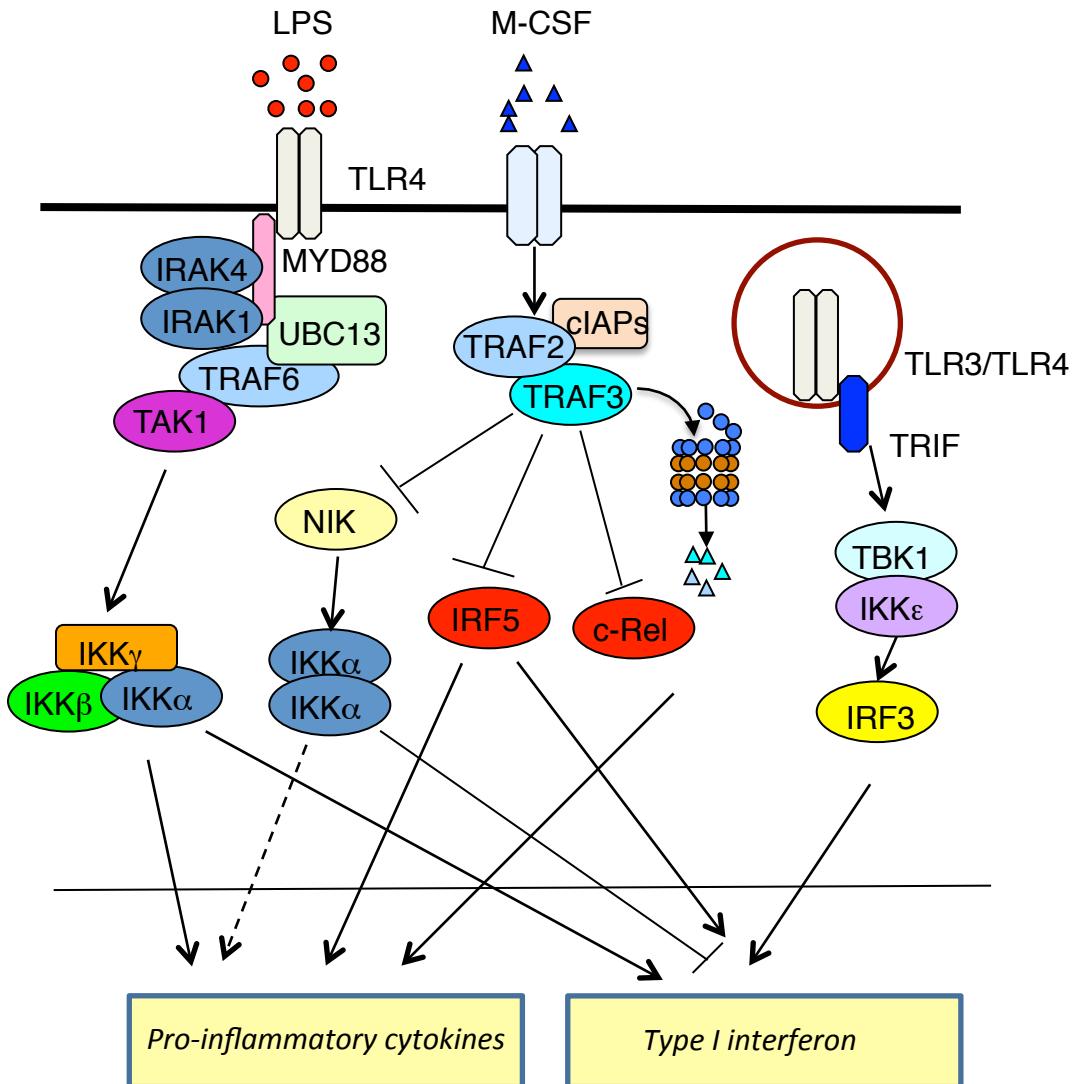
Supplementary Figure 4. TRAF3 deficiency does not affect myeloid cell differentiation but promotes TLR-stimulated proinflammatory gene expression in BMDMs. (a) Flow cytometry analysis of the percentage (numbers in quadrants) of neutrophils (CD11b⁺Gr-1⁺) and macrophages (CD11b⁺F4/80⁺) in the bone marrow of WT or TRAF3-MKO mice, presented as a representative FACS plot (left) and a summary graph (right). (b) qRT-PCR analysis of LPS- and poly(I:C)-stimulated BMDMs generated from the bone marrows of TRAF3^{+/+Cre^{ER} or TRAF3^{f/fCre^{ER} mice in the presence of the Cre inducer tamoxifen. Data are presented as fold relative to the Actb mRNA and representative of three or more independent experiments. Error bars represent SD, and differences between experimental and control groups were determined by Student's *t* test. *P < 0.05; **P < 0.01.}}



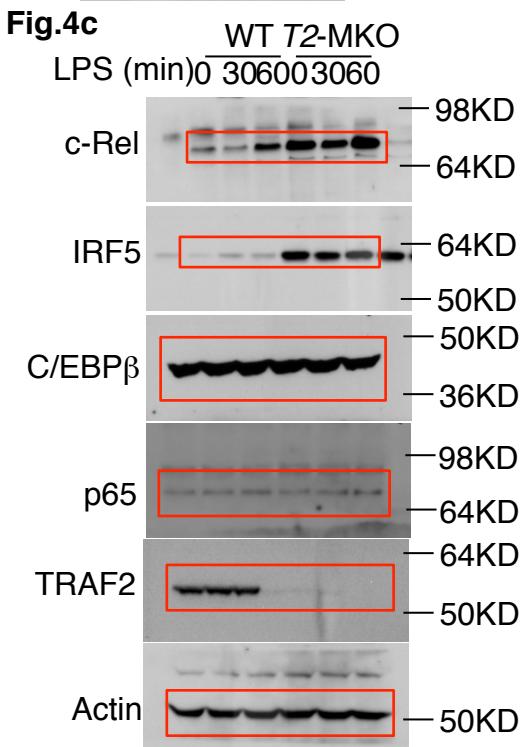
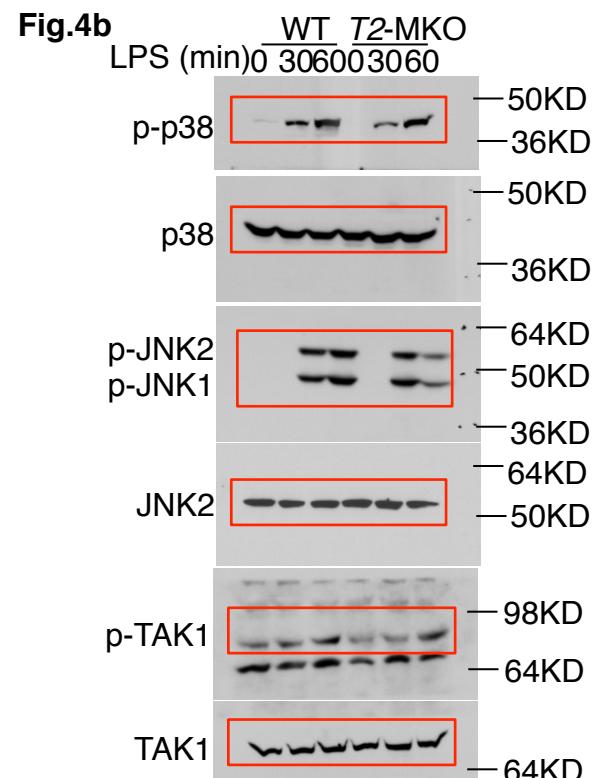
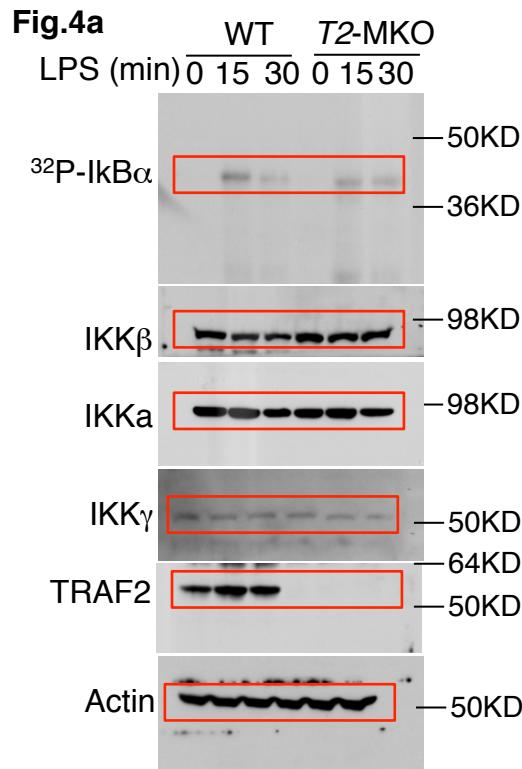
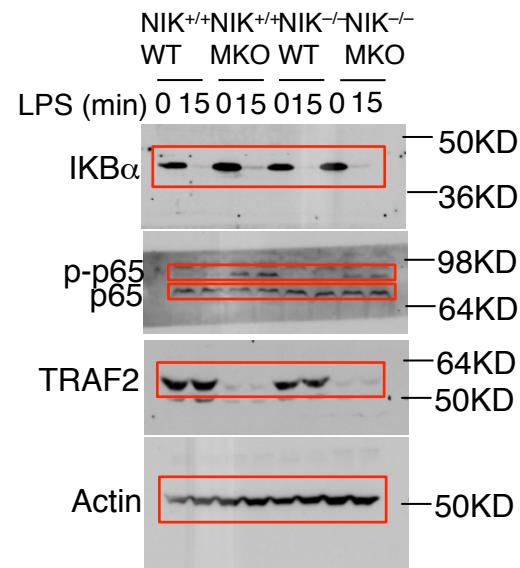
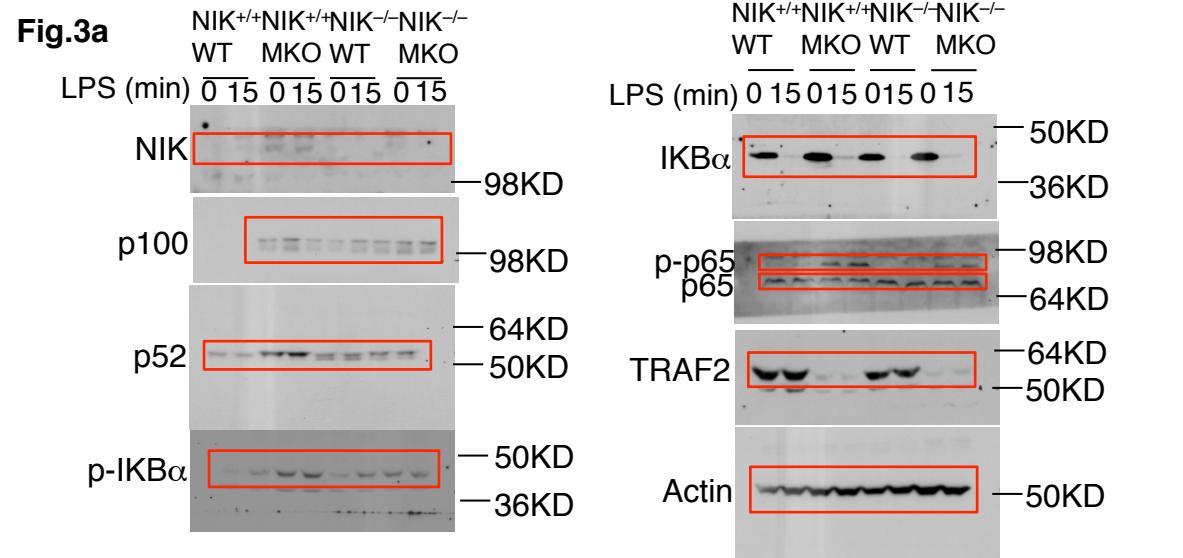
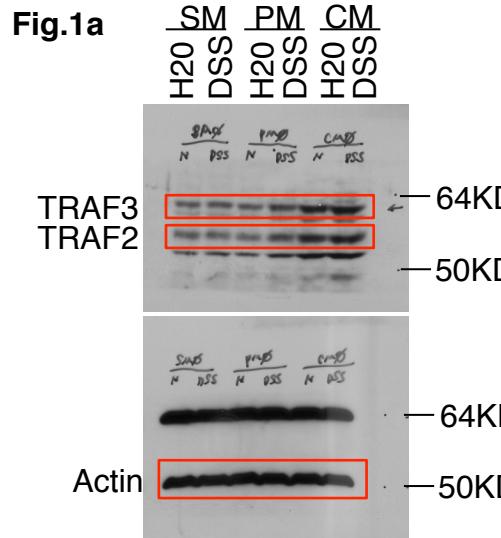
Supplementary Figure 5. TRAF3, but not TRAF2, directly interacts with c-Rel and IRF5. HEK293 cells were transfected with the indicated expression vectors. Cell lysates were subjected to IP using anti-c-Rel or anti-IRF5 followed by IB to detect precipitated HA-TRAF2 and HA-TRAF3 (top panel). Cell lysates were also directly analyzed by IB (bottom three panels). Data are representative of three independent experiments.



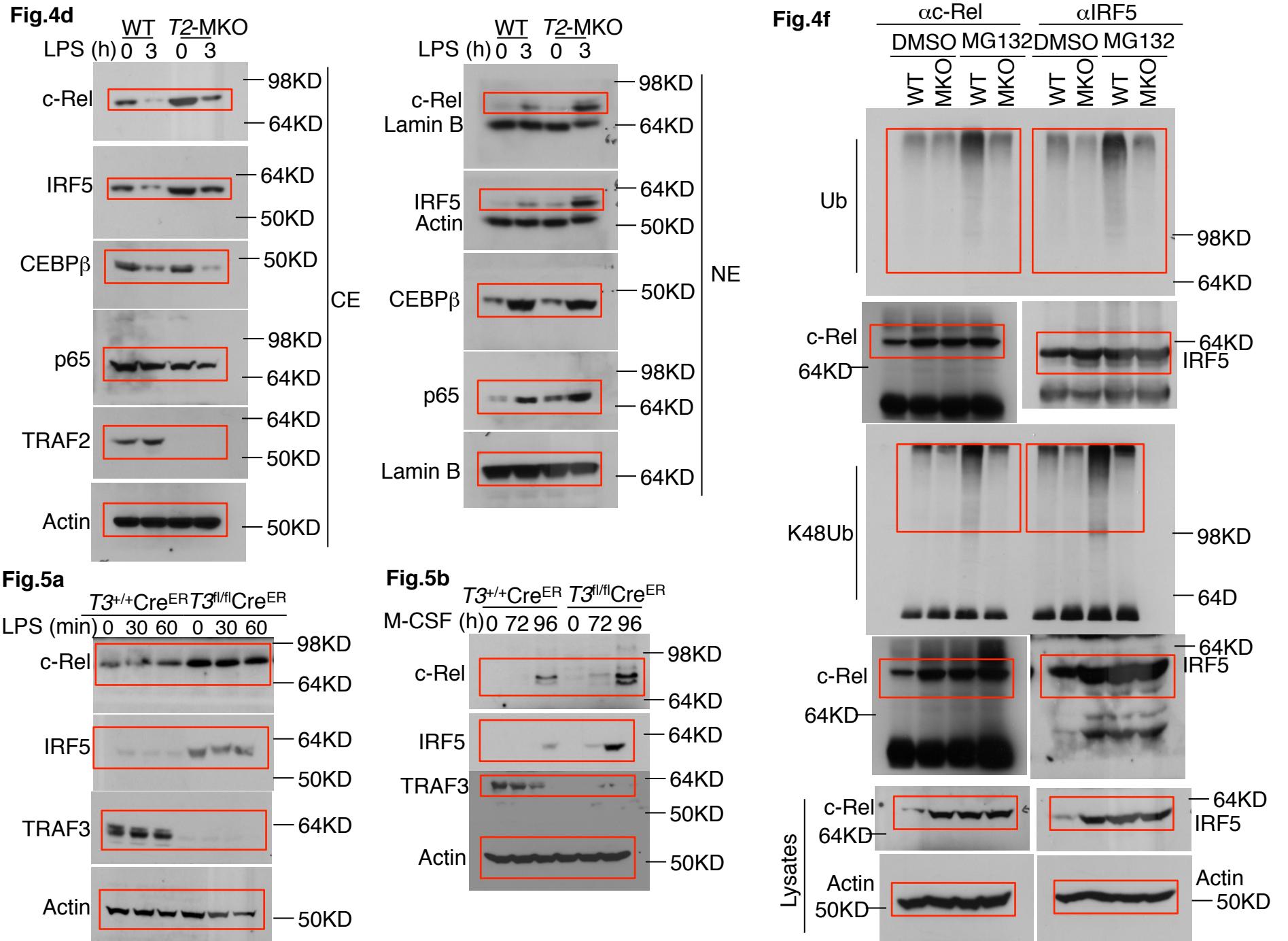
Supplementary Figure 6. Myeloid cell-specific ablation of TRAF2 enhances the frequency of tumor-infiltrating T cells. Flow cytometric analysis of CD4⁺ T cells, CD8⁺ T cells, macrophages, and MDSC in tumors of WT and *TRAF2*-MKO mice on day 18 after B16 cell inoculation. Data are presented as cell number per gram of tumor tissues. The error bars represent standard deviation (SD). Differences between experimental and control groups were determined by Student's t test. *P < 0.05; **P < 0.01.



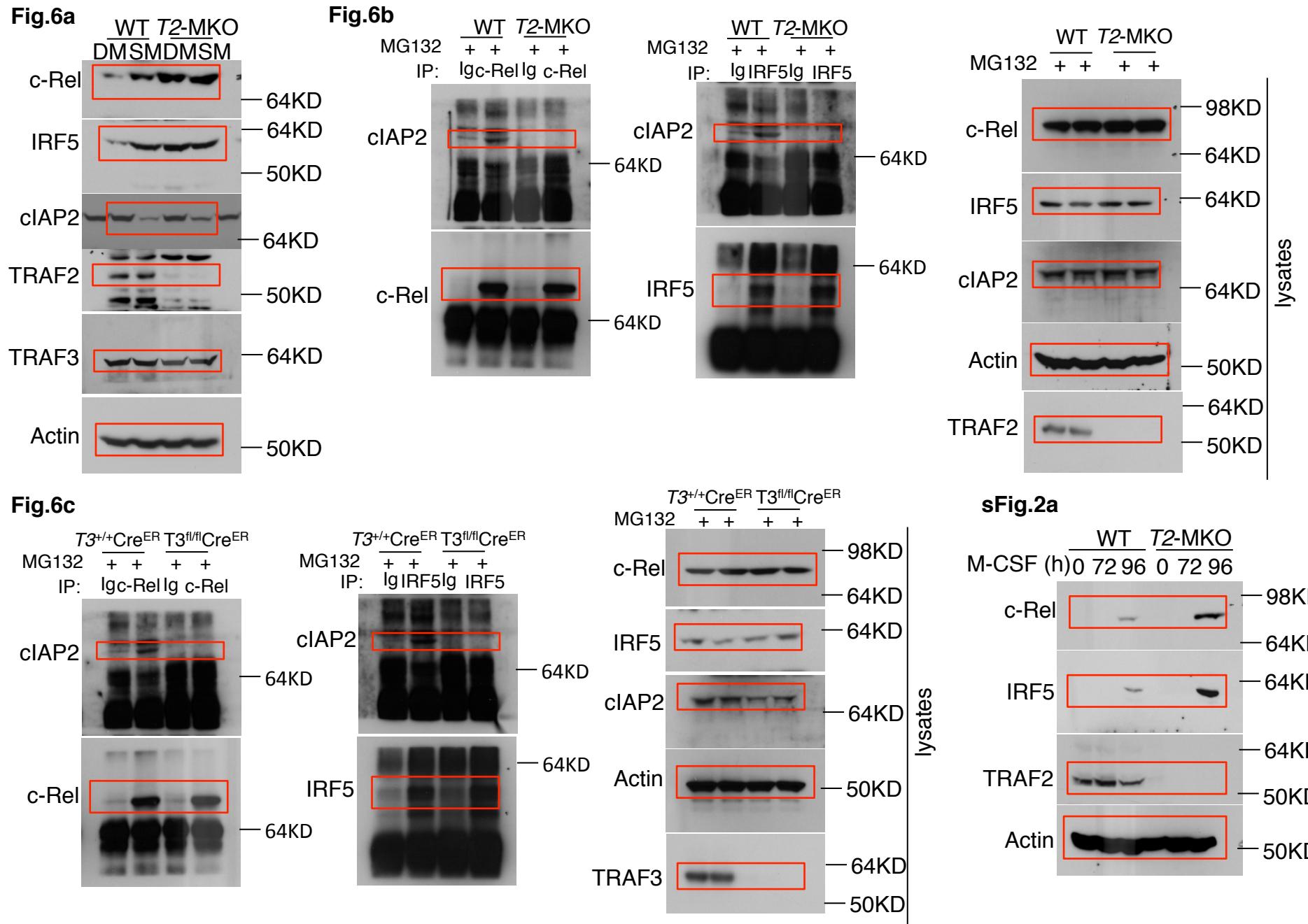
Supplementary Figure 7. A model of TRAF2 function in TLR4 signaling. TRAF2 bridges cIAP to TRAF3 to form an E3 ubiquitin ligase complex, in which TRAF3 recognizes three different substrates, NIK, IRF5, and c-Rel. IRF5 and c-Rel are crucial for mediating TLR-stimulated proinflamamtoy cytokine expression, although NIK-IKK α pathway may also play a role. Based on previous work, the IRF5 and NIK-IKK α pathways also regulate type I interferon induction in a positive and negative manner, respectively. Based on the present and previous studies, the M-CSF receptor recruits the TRAF3-TRAF2-cIAP complex and induces degradation of TRAF3 (as well as TRAF2), thereby interrupting the E3 function of this complex in the control of NIK, IRF5, and c-Rel.



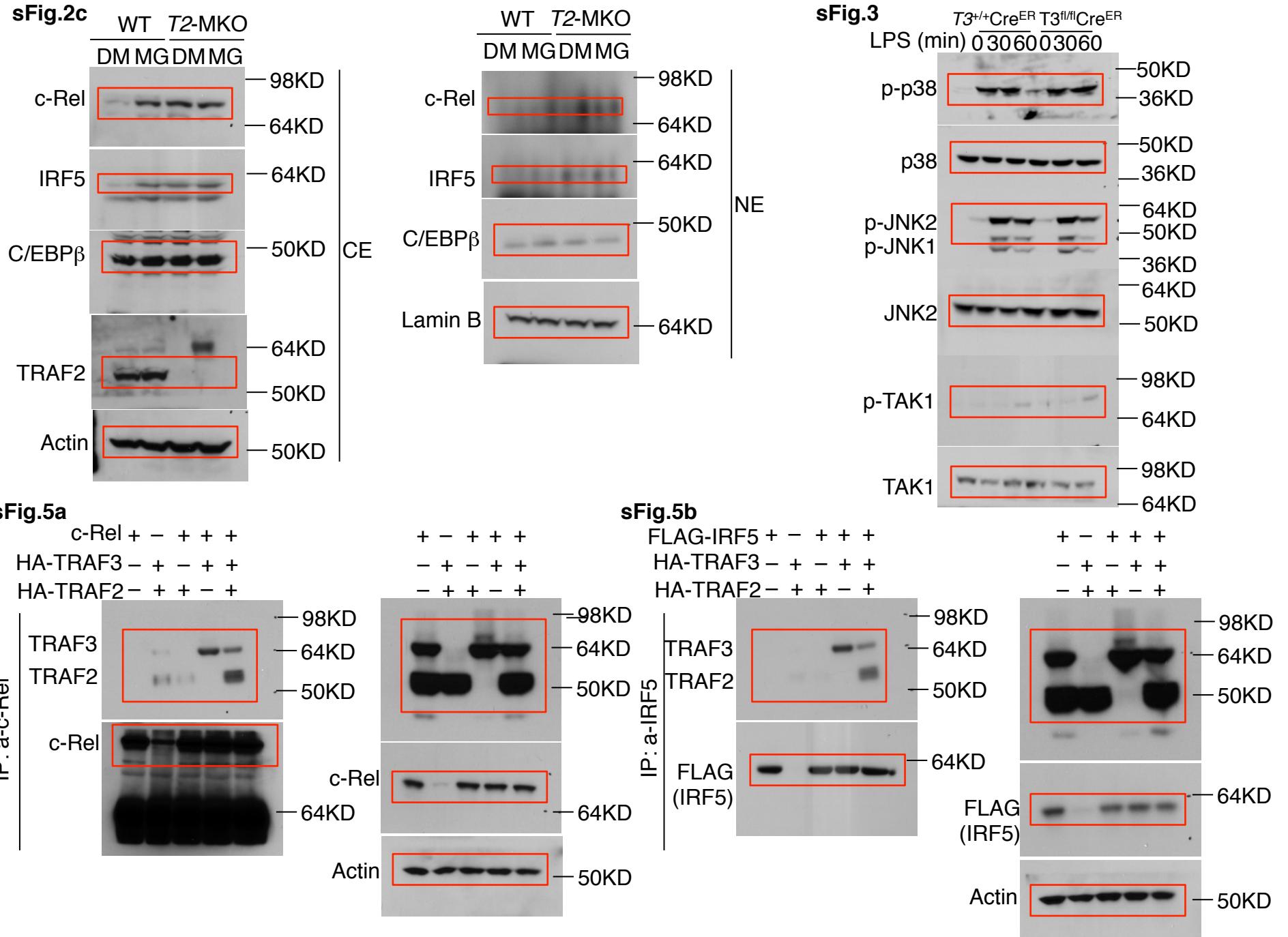
Supplementary Figure 8. Full scans of blots for figures 1a, 3a, and 4a-4c.



Supplementary Figure 9. Full scans of blots for figures 4d, 4f, 5a, and 5b.



Supplementary Figure 10. Full scans of blots for figure 6a-c and supplementary figure 2a.



Supplementary Figure 11. Full scans of blots for supplementary figures 2c, 3, 5a, and 5b.

Supplementary Table 1. The gene-specific primers used in qRT-PCR experiments

Type	Gene	Forward primer	Reverse primer
QPCR	<i>Il1b</i>	AAGCCTCGTGCTGTCGGACC	TGAGGCCAAGGCCACAGGT
QPCR	<i>Il6</i>	CACAGAGGATACCACTCCAAACA	TCCACGATTCCCAGAGAACAA
QPCR	<i>Tnf</i>	CATCTTCTCAAATTGAGTGACAA	CCAGCTGCTCCTCCACTTG
QPCR	<i>Il12p35</i>	ACTAGAGAGACTTCTTCCACAACAAGAG	GCACAGGGTCATCATCAAAGAC
QPCR	<i>Il12p40</i>	GGAGACACCAGCAAAACGAT	TCCAGATTCAAGACTCCAGGG
QPCR	<i>Il23p19</i>	GCCAAGAAGAC CATTCCGA	TCAGTGCTACAATCTCTCAGAGGACA
QPCR	<i>Il10</i>	CCAGAGCCACATGCTCCTAGA	GGTCCTTGTTGAAAGAAAGTCTTC
QPCR	<i>Nos2</i>	GTGGTGACAAGCACATTGG	AAGGCCAACACACAGCATACC
QPCR	<i>Tra2</i>	TGGCTGGCCGCATACC	TGTAGCCGTACCTGCTGGTGTA
QPCR	<i>Traf3</i>	CAAGTGCAGCGTTCAGACTC	GCAGCCATAGCGCTAAAAC
QPCR	<i>Rel</i>	CAACTGGAGAAGGAAGATTCA	TGGAACTCCTGAAGACCTG
QPCR	<i>Irf5</i>	TGCCTTGACGGACCTAGAG	AGGGCCAAAGAGTTCCACTT
CHIP	<i>IL6 (kB sites)</i>	TTTCCAATCAGCCCCACC	CAGAATGAGCTACAGACATCCC
CHIP	<i>IL1b (kB sites)</i>	GTTCCGCACATCCTGACTTA	CAATTGTGCAGATGGTGTCAA
CHIP	<i>IL12a (kB sites)</i>	ACGCACTTGTCCTTGAGATG	CTGACCTTGGGAGACACATT
CHIP	<i>IL12b (kB sites)</i>	CATTCCTCTAACCTGGGATTTC	CTGCTCCTGGTGCTTATACT
CHIP	<i>Arg1 (STAT+CEBP sites)</i>	TAGGAAGTGAGGCATTGTTCAG	GCACAACTCACGTACAGACA